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(54) Title: A METHOD OF AND DEVICE FOR DETERMINING OVULATION IN MAMMALS

(57) Abstract

The present invention relates to a method of determining the ovulation state of a mammal. The method includes obtaining a blood sample which includes a hormone of interest from the mammal, contacting the blood sample with a medium which includes a first antibody specific for the hormone of interest, where the hormone of interest binds to the first antibody, and contacting the blood sample with a labeled second antibody specific for the hormone of interest, where the hormone of interest binds to the labeled second antibody. The first antibody, the labeled second antibody, and the hormone of interest form a complex which gives an indication of the ovulation state of the mammal. The method also includes observing the indication to determine the ovulation state of the mammal. The present invention also relates to a medium which includes an antibody which is used in the present invention.

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A METHOD OF AND DEVICE FOR DETERMINING OVULATION IN MAMMALS

Cross-Reference to Related Applications

Applicants hereby claim priority based on two earlier filed U.S. provisional patent applications, Serial Nos. 60/122,658 (filed March 3, 1999) and 60/102,199 (filed September 29, 1998), both of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to a method of and device for determining ovulation in mammals. In particular, the present invention is directed to determining the onset of ovulation based upon the detection of hormones in a blood sample of a mammal.

BACKGROUND OF THE INVENTION

Ovulation in mammalian females is orchestrated by a number of hormones, rising and falling in turn. Specifically, luteinizing hormone ("LH"), follicle-stimulating hormone ("FSH"), progesterone, estradiol, and estrogen are essential hormones whose concentrations fluctuate throughout the female sex cycle. Hormone peaks and troughs are detectable within the blood, thus providing potential for ovulation prediction.

Of these hormones, LH has the most pronounced surge. Immediately before ovulation across all

mammalian species, LH levels rise significantly. In almost all mammals, LH surges within a short, confined period before ovulation occurs. Therefore, LH-blood concentration is a reliable indicator for ensuing ovulation. LH has its primary effect on the receptors of the ovaries, culminating in the expulsion of an egg from the ovum.

FSH also varies its concentrations before ovulation. However, the rise in FSH concentration is slight and gradual. Therefore, precise species specific mechanisms are important to detect changes in FSH concentration. Additionally, FSH is not present in detectable levels in all mammals.

Progesterone levels generally increase after ovulation. Like FSH, progesterone exhibits moderate concentration differences, accentuated by a steady rise and fall. Progesterone production and secretion is stimulated by changing LH concentrations. LH's surge stimulates the cell activity, causing the granulosa cells of the ovary to begin progesterone production.

Another hormone essential for the female sex cycle is estrogen. Like the previously described hormones, estrogen experiences a rise and fall during the ovulatory process of a mammalian female. Some estrogen increases are initiated during the pre-ovulatory antral expansion of the ovum; during this portion of the cycle, increasing FSH blood concentrations cause estrogen levels to also rise. In cows, estrogen experiences a slight rise about a week after ovulation. In humans, this rise occurs several days prior to the ovum's release, but before the LH surge. In contrast, the steady increase of the production of progesterone in

primates and pigs is the cause of estrogen increases in these animals. Therefore, the point at which estrogen peaks during the female sex cycle is nearly species specific. However, estrogen's function during the menstrual cycle is more extensive.

Other methods for ovulation detection include a blood serum radioimmunoassay analysis, as first described by Sakai et al., "Evidence For Alterations in Luteinizing Hormone Secreted in Rhesus Monkeys with Normal and Inadequate Luten Phases Using Radioreceptor and Radioimmunoassay," Endocrinology, 104:1217-1225 (1979). However, a radioimmunoassay measures both beta subunits and LH hormones in the serum, therefore, it is not a reliable LH indicator. Selvaraj et al., "Development of an LH Receptor Assay Capable of Measuring Serum LH/CG in a Wide Variety of Species," J. Reproduction & Fertility, 98: 611-616 (1993) developed a radioreceptor assay measuring only LH-blood serum concentrations. This assay produced reliable results and was more cost effective, although the assay still required either a homogenized blood or urine serum for testing.

The most reliable test to determine the ovulation time period of a mammal is a clinical analysis of a blood sample. However, this procedure is expensive because it requires the assistance of a trained clinician and the drawn blood sample must be processed in a laboratory. Furthermore, because of the processing delay clinical testing requires, the predictive ovulation window is reduced, decreasing the probability of impregnation. An additional complication associated with this form of testing occurs if the female is not

experiencing an LH surge at the time of the blood test. Variables including irregular cycling, a condition known as *oligomenorrhoea*, will prevent a mammalian female from ovulating during an anticipated time frame. Therefore, testing must be repeated until ovulation is detected, potentially incurring considerable expenses.

SUMMARY OF THE INVENTION

The present invention relates to a method of and device for determining the ovulation state of a mammal. The method includes obtaining a blood sample which includes a hormone of interest from the mammal, and contacting a medium with the blood sample at a location on the medium having a first antibody specific for the hormone of interest. The hormone of interest binds to the first antibody. Next, medium is contacted with the blood sample at a location on the medium having a labeled second antibody specific for the hormone of interest, and the hormone of interest binds to the labeled second antibody. The first antibody, the labeled second antibody, and the hormone of interest form a complex which gives an indication of the ovulation state of the mammal. The method also includes observing the indication to determine the ovulation state of the mammal.

Another aspect of the present invention relates to a device which includes a first antibody immobilized at a first predetermined position on the device, where the first antibody is specific for a mammal hormone of interest. In an embodiment of the present invention, a blood sample is applied to a reagent impregnated in the

test strip. The sample is allowed to permeate along the test strip and progress into or through a detection zone on the test strip. In a particular embodiment of the present invention, the test strip includes a specific ELISA monoclonal antibody based assay for LH wherein a drop of whole blood is applied to the test strip, allowed to react with the primary monoclonal antibody contained in a porous matrix, washed with a secondary antibody linked to a chromophore, rinsed and the extent of the resulting color is measured, the resulting color being proportional to the amount of LH in the sample. When the species LH differs, a species-specific antibody or fluorescence may be developed for each mammal.

The resulting color can be measured by visual comparison to a color chart. The visual comparison to a color chart provides an approximation of the LH level. Various alternative means can be employed for visually reading the strip, including assessing color differences from a solid surface, for example by reflectance spectrophotometric means.

By using the method and device of the present invention, fast, reliable, and inexpensive testing is available to determine whether a female mammal is ready for breeding. Thus, mammal breeders can more accurately determine when to mate the mammal. In the pig breeding industry, for example, this should result in savings of up to hundreds of millions of dollars per year.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a top plan view of a device according to the present invention;

Figure 1B is a cross-sectional side view of the device shown in Figure 1A taken along line 1B--1B;

Figure 2 is a schematic of a method according to the present invention; and

Figure 3 illustrates a side view of a liquid phase assay of an embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Although the embodiment of the present invention described below is specifically directed to female pigs, the present invention may be directed to any female mammal in which there is a detectable hormone surge during the mammal's cycle prior to ovulation. Such mammals include humans, domestic mammals such as, for example, horses, cows, sheep, dogs, and wild mammals, such as for example, tigers, lions, bears, primates including monkeys and baboons or other mammals typically found in zoos. In particular, the present invention is particularly useful for mammals in which current methods to detect ovulation are expensive, inaccurate, and time consuming.

A method according to the present invention includes obtaining a blood sample having a hormone of interest from the pig, contacting the blood sample with a medium which includes a first antibody specific for the hormone of interest, where the hormone of interest binds to the first antibody. The method also includes contacting the blood sample with a labeled second antibody specific for the hormone of interest, where the hormone of interest binds to the labeled second antibody. The first antibody, the labeled second

antibody, and the hormone of interest form a complex which gives an indication of the ovulation state of the pig. The method also includes observing the indication to determine the ovulation state of the pig.

Another aspect of the present invention relates to a test device which includes a first antibody immobilized at a first predetermined position on the test device, where the first antibody is specific for a pig hormone of interest. Preferably, the medium of the present invention is a test strip or a test apparatus having test cells.

Ovulation in female mammals is preceded by a hormone surge (U.S. Patent No. 5,460,976 to O'Connor; U.S. Patent No. Re. 32,557 to Chatterton; Anderson et al., "Luteinizing Hormone Levels in Pig, Cow and Rat Blood Plasma During the Estrous Cycle," Endocrinology, 78(5):976-82 (1966)("Anderson"); Pant et al., "Concentration of Oestradiol, Progesterone, Luteinizing Hormone and Follicle-Stimulation Hormone in the Jugular Venous Plasma of Ewes During the Oestrous Cycle," J. Endocr., 73:247-55 (1977) ("Pant"); Guerin et al., "Use of An Immunoenzymatic Assay to Detect the Luteinizing Hormone Peak in Bitches," J. Reproduction & Fertility Supp., 51:277-81 (1997)("Guerin"); and Britt et al., "Roles of Estradiol and Gonadotropin-Releasing Hormone in Controlling Negative and Positive Feedback Associated with the Luteinizing Hormone Surge in Ovariectomized Pigs," Biol. of Reproduction, 45:478-85 (1991) ("Britt"), which are hereby incorporated by reference). Of particular interest is luteinizing hormone ("LH"), which surges approximately 36-40 hours prior to ovulation in pigs (Britt). Thus, measurement of the

levels of LH will be an accurate indication of the ovulation state of the pig.

As used herein, "ovulation state" refers to either a positive ovulation state or a negative ovulation state. "Positive ovulation state" refers to that portion of the cycle 40 hours prior to through 24 hours after ovulation of the follicle from the ovary of the mammal. "Negative ovulation state" refers to that time period outside of the positive ovulation state.

One embodiment of a device according to the present invention is a test strip 10 as shown in Figures 1A and 1B. In this embodiment, the test strip 10 is a solid phase test strip 10 having a support base 12 made of a polymer, such as styrene, polystyrene, or polycarbonate, with polystyrene being particularly preferred. The support base 12 gives the test strip 10 structural integrity.

The test strip 10 includes a first antibody 14, which is specific for a hormone of interest in the blood sample of the pig. The first antibody 14 is immobilized on the test strip 10 at a first predetermined position 17. The first antibody 14 is immobilized in a porous nonreactive carrier matrix 16. Such matrices are commonly used for nucleic acid and protein binding. Examples of materials suitable for the carrier matrix 16 include nitrocellulose and nylon. When the carrier matrix 16 is nitrocellulose, antibodies can be directly immobilized onto the carrier matrix 16 without the need of a chemical treatment. For other types of matrices, immobilization can be accomplished by techniques such as treatment with cyanogen bromide and carbonyldiimidazole known to those skilled in the art.

The first antibody 14 is specific for a hormone of interest in the blood sample of the pig. As used herein, "specific" means an antibody which has a high binding affinity for the hormone of interest. This means an antibody which binds to the hormone of interest under high stringency conditions.

The first antibody 14 is a monoclonal antibody, a polyclonal antibody, or fragments thereof. A monoclonal antibody is preferred. The particular choice of the antibody will depend on the hormone of interest. For example, for detection of luteinizing hormone ("LH"), antibodies which have specific binding affinity to LH are immobilized on to the carrier matrix 16.

The first antibody 14 may be supplied by antibody suppliers known to those skilled in the art. Alternatively, monoclonal antibody production may be effected by techniques well-known in the art. Generally, the monoclonal antibody production process involves obtaining immune cells (lymphocytes) from the spleen of a mammal (e.g., mouse) which has been previously immunized with the antigen of interest either *in vivo* or *in vitro*. The antibody secreting lymphocytes are then fused with (mouse) myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The resulting fused cells, or hybridomas, are cultured, and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned and grown either *in vivo* or *in vitro* to produce large quantities of antibody. A description of the theoretical basis and practical

methodology of fusing such cells is set forth in Kohler et al., Nature, 256:495 (1975), and is hereby incorporated by reference.

Mammalian lymphocytes are immunized by *in vivo* immunization of the animal. Such immunizations are repeated as necessary at intervals of up to several weeks to obtain a sufficient titer of antibodies. Following the last antigen boost, the animals are sacrificed and spleen cells removed.

Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, for example, by using polyethylene glycol for the fusing agents (Milstein et al., Eur. J. Immuol., 6:511 (1976), which is hereby incorporated by reference). This immortal cell line, which is preferably murine, but may also be derived from cells of other mammalian species, including but not limited to rats and humans, is selected to be deficient in enzymes necessary for the utilization of certain nutrients, to be capable of rapid growth, and to have good fusion capability. Many such cell lines are known to those skilled in the art, and others are regularly described.

Procedures for raising polyclonal antibodies are also well known. Typically, such antibodies can be raised by administering a protein or polypeptide subcutaneously to New Zealand white rabbits which have first been bled to obtain pre-immune serum. The antigens can be injected at different sites, where each injected material will contain synthetic surfactant adjuvant pluronic polyols, or pulverized acrylamide gel containing the protein or polypeptide after SDS-

polyacrylamide gel electrophoresis. The rabbits are then bled two weeks after the first injection and periodically boosted with the same antigen. A sample of serum is collected after each boost. Polyclonal antibodies are then recovered from the serum by affinity chromatography using the corresponding antigen to capture the antibody. This and other procedures for raising polyclonal antibodies are disclosed in Harlow et al., Antibodies; A Laboratory Manual (1988), which is hereby incorporated by reference.

In addition to utilizing whole antibodies, the present invention encompasses use of binding portions of such antibodies. Such binding portions include Fab fragments, $F(ab')_2$ fragments, and Fv fragments. These antibody fragments can be made by conventional procedures, such as proteolytic fragmentation procedures as described in Goding, Monoclonal Antibodies: Principles and Practice, pp. 98-118 (N.Y. Academic Press 1983), which is hereby incorporated by reference.

The hormone of interest in the present invention includes luteinizing hormone, follicle-stimulation hormone, estradiol, progesterone, or estrogen. As discussed above, the levels of these hormones rise and fall throughout the estrous cycle of a mammal in relation to the mammal's ovulation state. Therefore, the levels of any of these hormones is indicative of a point in the mammal's estrous cycle, and can be related to the mammal's ovulation state. Preferably, the hormone of interest is LH.

The second antibody 18 is independent from the test strip 10 to be added at a later stage, as described below or, alternatively, is detachably located on the

test strip 10 at a second predetermined position 19 distal to the first predetermined position 17 as shown in Figures 1A and 1B. The second antibody 18 is detachably attached to the test strip 10 such that, when the test strip 10 is contacted with the blood sample, the second antibody 18 will propagate along the test strip 10 toward the carrier matrix 16 via a wick section 22. The wick section 22 may be a paper material such as filter paper suitable for allowing the blood sample to propagate through the wick section 22 to reach the carrier matrix 16 and the first predetermined position 17.

The second antibody 18 is labeled. Examples of labels useful in the present invention are radiolabels such as ^{131}I, ^{111}In, ^{123}I, ^{99}mTc, ^{32}P, ^{125}I, ^{3}H, ^{14}C, and ^{188}Rh, fluorescent labels such as fluorescein and rhodamine, chemiluminescers such as lucifein, enzymatic markers such as peroxidase or phosphatase, and chromophores. A UV photoreactive dye may or may not be used to label the second antibody 18. Procedures for labeling antibodies with labels of these types are described in Wensel et al., Radioimmunoimaging and Radioimmunotherepy, Elsevier, New York (1983); Colcher et al., Meth. Enzymol., 121: 802-16 (1986); Goding et al., Monoclonal Antibodies: Principles and Practice, pp. 124-26 (1983) ("Goding"); Hunter et al., Nature, 144:945 (1962); David et al., Biochemistry, 13:1014-21 (1974); Grenwood et al., Biochem J., 89:114-23 (1963); Marchalonis, Biochem J., 113:299-305 (1969); and Morrison et al., Immunochemistry, 289-297 (1971), which are hereby incorporated by reference. Preferably, the antibody is labeled with a chromophore. Suitable chromophores are

described by Stryker, Science, 162:526 (1968) and Brand et al., Ann Review of Biochem., 41:843-68 (1972), which are hereby incorporated by reference.

In an alternative embodiment, a third antibody 20 is immobilized on the test strip 10 at a third predetermined position 21. The third predetermined position 21 is located on a carrier matrix 16 between the first predetermined position 17 and the second predetermined position 19. The third antibody 20 is a capture antibody which will capture the second antibody 18. The third antibody 20 is immobilized on the test strip 10 in the carrier matrix 16 in the same manner as described above for the first antibody 14.

The test strip 10 preferably includes a filter 23. Preferably, the filter 23 is a removable glass fiber filter, as known to those skilled in the art. The filter 23 operates to remove cells and particulate matter from the whole blood sample allowing blood serum to pass through the filter 23. The hormone of interest is too small to be captured by the filter 23 and, therefore, passes through the filter 23.

Preferably, the test strip 10 is blocked with a blocking agent prior to immobilization of the first antibody 14, the second antibody 18 and the third antibody 20. Blocking agents may include bovine serum albumin, diluted serum, non-fat dry milk, and casein. Blocking agents prevent binding of unwanted compounds to the test strip 10.

In use, a sample S of whole blood from a mammal whose LH blood level is to be analyzed is first obtained from the subject. Typically, the blood sample is obtained from a vasculature area of the subject, such as

the ear, by means of a pin prick. The sample S, which may be as little as a drop, is then applied to the test strip 10 and allowed to react (typically 30 seconds to several minutes).

As shown in Figure 2, the test strip 10 is contacted (step 50) with the sample S. Preferably, the sample S is provided to the test strip 10 at an end of the test strip 10 distal to the first antibody 14. Preferably, the sample S is provided to the test strip 10 at the second predetermined position 19. Optionally, the sample S is filtered by a filter 23 such that cells and particulate matter are removed from the sample S to result in a blood serum S' passing through the filter 23. The hormone of interest will be present in the serum S'.

As discussed above and as shown in Figure 2, in one embodiment the labeled second antibody 18 is located on the test strip 10 proximate to the area where the test strip 18 is contacted with the sample S. Alternatively, in a second embodiment, the labeled second antibody 18 is added independently to the test strip 10 after the sample S has been applied to the test strip 10. Preferably, the labeled second antibody 18 is present in a solution which is applied by an absorbent paper to the test strip 10. The solution having the labeled second antibody 18 provides additional liquid to assist the propagation (step 55) of the sample S, or serum S', as the case may be, along the test strip 10.

The hormone of interest H present in the sample S, such as LH, will bind to the labeled second antibody 18 in the manner as described above to form a first complex C, as shown in Figure 2. The first complex C propagates

up the test strip 10 to the first predetermined position 17 where the first antibody 14 is located. The hormone of interest H will bind to the first antibody 14 in the manner described above to form a second complex C' of the first antibody 14, the labeled second antibody 18, and the hormone of interest H.

If the level of the hormone of interest H in the sample S is sufficiently high, enough hormone of interest will bind to the labeled second antibody 18 to achieve a detectable level of the second complex C' to produce (step 60) a colored band B at the first predetermined position 17, as shown in Figure 2. For example, when the labeled second antibody 18 is labeled with a chromophore, the second complex C' will be detectable as a concentrated band of color at the first predetermined position 17. As used herein, "detectable" means visible to an ordinary person using the naked eye. This indicates a positive state of ovulation. If there is a low level of the hormone of interest H present in the sample S, there will not be a sufficient amount of the labeled second antibody 18 present in the complex C' to be detectable. This indicates a negative state of ovulation.

As the sample S travels over the third predetermined position 21, an indication will be given that the test is proceeding properly. In particular, a thin band of color will appear at the third predetermined position 21 to indicate that the sample S has passed through the third predetermined position 21. Preferably, this indication is provided by the third antibody 20, which is a capture antibody that captures

the second antibody 18 as it passes over the third predetermined position 21.

In an alternate embodiment, as shown in Figure 3, the medium is a test apparatus as described in U.S. Patent Application Serial No. 09/208,648, the disclosure of which is hereby incorporated by reference. Preferably, the test apparatus is a liquid phase enzyme-linked immunosorbant assay ("ELISA") test plate 30 which includes liquid holding cells 32. One of the liquid holding cells 32 is designated as a reference or blank cell 34 and one or more of the other holding cells 32 are designated as test cells 36. Holding cells 32 are covered by a removable filter 38. In one embodiment, each test cell 36 contains a first antibody 14 bound to its walls. A sample of whole bloods from a mammal is obtained and applied to each test cell 36 and to the blank cell 34. The cellular matter in the blood is filtered by a removable filter 38. The serum S' that passes through the filter 38 is allowed to react with the first antibody 14 bound to cell walls of the test cells 36, and allowed to encounter the blank cell 34 containing blocked substrate without the first antibody 14. The filter 38 is then removed and the test cells 36 are washed to remove unreacted material. The second antibody 18, specific for the conjugated first antibody or for the initial analyte, labeled with a chromophore, fluorescent, or luminescent complex (as described above) is applied to the test cells 36. The test cells 36 are then rinsed to remove the uncombined secondary complex and the intensity of the color, fluorescence or luminescence is measured. The intensity of the color, fluorescence, or luminescence is indicative of the

amount of the chromophore, fluorescent, or luminescent complex, respectively, present in the sample which, in turn, is indicative of the amount of analyte in the blood sample S.

In the embodiments described above, the color may be measured by comparison to a color chart to determine the LH level. In addition, various alternative means can be employed for visually reading the strip. This would include various means for assessing color differences from a solid surface. One illustrative embodiment is described in U.S. Patent Application No. 09/208,648, the disclosure of which is hereby incorporated by reference. Another illustrative embodiment is described in U.S. Provisional Patent Application No. 60/102,199 filed September 29, 1998 the disclosure of which is hereby incorporated by reference.

The present invention does not require blood preparation work. Whole blood samples are taken from the mammalian female, thus, minimal training is required of the user. In addition, the test is cost efficient.

There are several additional advantages for the method of the present invention. For example, the invention will be beneficial to those breeders who use artificial insemination ("AI") techniques to promote conceptions. AI is an expensive procedure, costing nearly $19 per attempt. In contrast, monoclonal antibody blood testing as described herein is estimated to cost only $2 per test, a savings of nearly 90%. Therefore, the invention could be used in conjunction with AI techniques to maximize the advantages of the latter, eliminating expensive, failed inseminations. Thus, the invention will save the animal breeding

industry substantial amounts. For example, in 1998 it is estimated that LH testing will save swine AI breeders $252 million; 2002, it is predicted that these savings will reach $410 million.

The present invention also has zoological significance. In order to ensure a diverse gene pool, captive species are often mated with animals from other zoos. However, this procedure is complicated and expensive. Therefore, particularly with large animals, it is important that the female is fertile at the time of insemination or mating. The method of the present invention, therefore, will be a useful aid in determining the fertility of the mammal, aiding in successful impregnation at minimal additional cost.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for illustration and variations can be made by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

What is Claimed:

1. A method of determining the ovulation state of a mammal comprising:

obtaining a blood sample comprising a hormone of interest from the mammal;

contacting a medium with the blood sample at a location on the medium having a first antibody specific for the hormone of interest, whereby the hormone of interest binds to the first antibody;

contacting the medium with the blood sample at a location on the medium having a labeled second antibody specific for the hormone of interest, whereby the hormone of interest binds to the labeled second antibody, wherein the first antibody, the labeled second antibody, and the hormone of interest form a complex which gives an indication of the ovulation state of the mammal; and

observing the indication to determine the ovulation state of the mammal.

2. The method according to claim 1, wherein the step of contacting the medium with the blood sample at the location having the labeled second antibody occurs prior to the step of contacting the medium with the blood sample at the location on the medium having the first antibody.

3. The method according to claim 1, wherein the medium further comprises the labeled second antibody.

4. The method according to claim 1, wherein the labeled second antibody is labeled with a label selected from the group consisting of a fluorescent label, a radiolabel, and a chromophore.

5. The method according to claim 4, wherein the label is a chromophore.

6. The method according to claim 1, wherein the indication is a positive indication.

7. The method according to claim 6, wherein the positive indication comprises a concentrated complex comprising the labeled second antibody, the first antibody, and the hormone of interest, wherein the concentrated complex is detectable.

8. The method according to claim 7, wherein the concentrated complex is detectable as a band of color at a predetermined position on the medium.

9. The method according to claim 1, wherein the hormone of interest is selected from the group consisting of luteinizing hormone, estradiol, follicle-stimulating hormone, progesterone, and combinations thereof.

10. The method according to claim 9, wherein the hormone of interest is luteinizing hormone.

11. The method according to claim 10, wherein the first antibody is a monoclonal antibody.

12. The method according to claim 11, wherein the labeled second antibody is a monoclonal antibody.

13. The method according to claim 1 further comprising a step of filtering the blood sample after the step of obtaining and prior to the contacting steps.

14. The method according to claim 1 further comprising a step of diluting the blood sample after the step of obtaining and prior to the contacting steps.

15. The method according the claim 1, wherein the medium is a strip.

16. The method according to claim 15, wherein the strip comprises nitrocellulose or nylon.

17. The method according to claim 1, wherein the medium further comprises a third antibody located at a predetermined location, wherein a portion of the hormone of interest binds to the third antibody to give a positive indication.

18. The method according to claim 1, wherein the mammal comprises humans, pigs, cows and horses.

19. The method according to claim 18, wherein the mammal is a human.

20. The method according to claim 18, wherein the mammal is a pig.

21. The method according to claim 18, wherein the mammal is a horse.

22. The method according to claim 18, wherein the mammal is a cow.

23. The method according to claim 1, wherein the mammal comprises wild animals including primates, lions, tigers and bears.

24. A device for determining the ovulation state of a mammal, comprising a first antibody immobilized at a first predetermined position on a medium, wherein the first antibody is specific for a mammal hormone of interest.

25. The device according to claim 24 further comprising a second antibody detachably immobilized at a second predetermined position on the medium, wherein the second antibody is specific for the mammal hormone of interest and wherein the second antibody is labeled.

26. The device according to claim 25, wherein the second antibody is labeled with a label selected from the group consisting of a fluorescent label, a radiolabel, and a chromophore.

27. The device according to claim 26, wherein the label is a chromophore.

28. The device according to claim 25 further comprising the mammal hormone of interest.

29. The device according to claim 28, wherein the mammal hormone of interest is selected from the group consisting of luteinizing hormone, estradiol, follicle-stimulating hormone, progesterone, and combinations thereof.

30. The device according to claim 29, wherein the mammal hormone of interest is luteinizing hormone.

31. The device according to claim 25, wherein the first antibody is a monoclonal antibody.

32. The device according to claim 31, wherein the second antibody is a monoclonal antibody.

33. The device according to claim 28, wherein the medium comprises a concentrated complex comprising the second antibody, the first antibody, and the mammal hormone of interest.

34. The device according to claim 25, wherein the medium is a strip.

35. The device according to claim 34, wherein the strip comprises nitrocellulose or nylon.

36. The device according to claim 25 further comprising a third antibody located at a third predetermined

location, wherein the third antibody is specific for the mammal hormone of interest.

37. A method of ascertaining an ovulation state of a mammalian female subject comprising the steps of:

obtaining a whole blood sample from the subject with a needle prick to a vasculature of the subject;

placing the blood sample on a test strip;

allowing the blood sample to effect a change in the test strip to provide an indication of LH concentration in the blood sample; and

comparing the indication of LH concentration to a comparison concentration to ascertain the ovulation state of the subject.

38. The method of claim 37, wherein the comparing step is performed by optically sensing the indication of LH concentration, and the comparison concentration is specific to a species of the subject.

39. The method of claim 37 further comprising a step of conveying information corresponding to the ascertained ovulation state of the subject to a user via an apparatus performing the comparing step.

40. The method of claim 37, wherein the test strip contains anti-human monoclonal antibodies bound to a matrix, and wherein a remaining matrix is blocked by a blocking reagent.

41. The method of claim 40, wherein a visible dye is used to label a secondary antibody, and the comparing step is performed by using a colormetric chart.

42. The method of claim 40, wherein the comparing step is performed by assessing color differences from a solid surface.

43. The method of claim 1, wherein a monoclonal antibody is added to the test strip after the blood sample binds to the test strip and exposed surfaces of the test strip have been blocked.

44. The method of claim 43, wherein a visible dye is used to label a secondary antibody, and the test strip is analyzed by comparison to a colormetric chart.

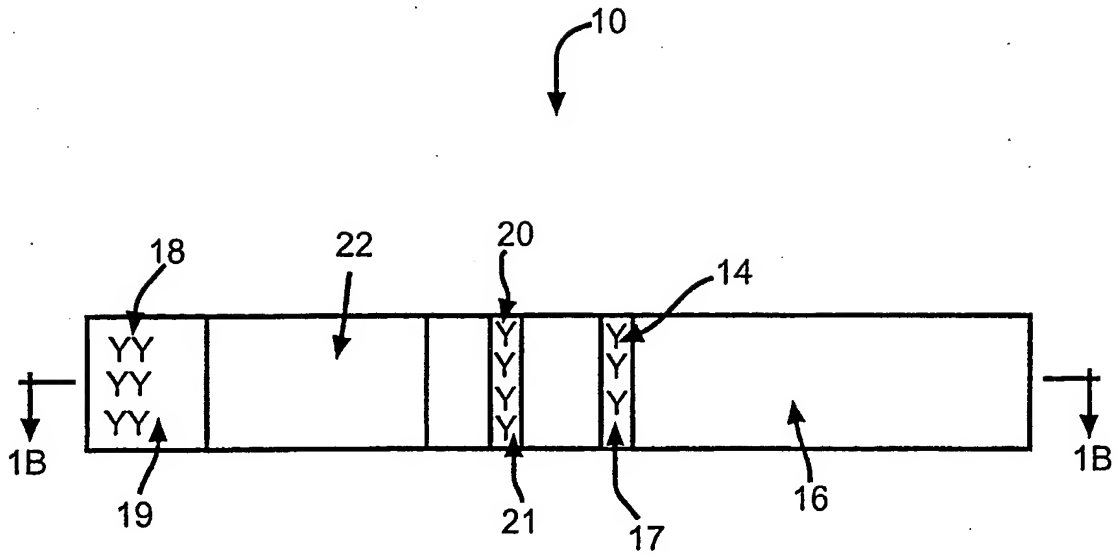
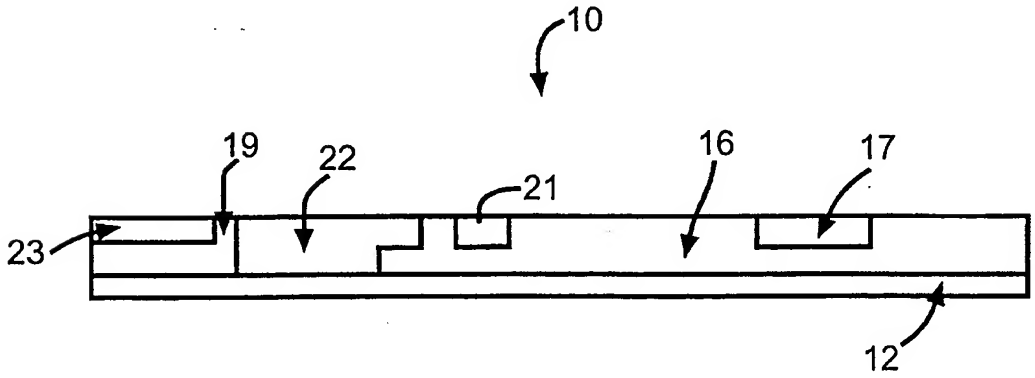

10
18
22
20
14
YY
YY
YY
Y
Y
Y
Y
Y
Y
Y
1B
1B
19
21
17
16

FIG.1A


10
19
22
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16
17
23
12

FIG.1B

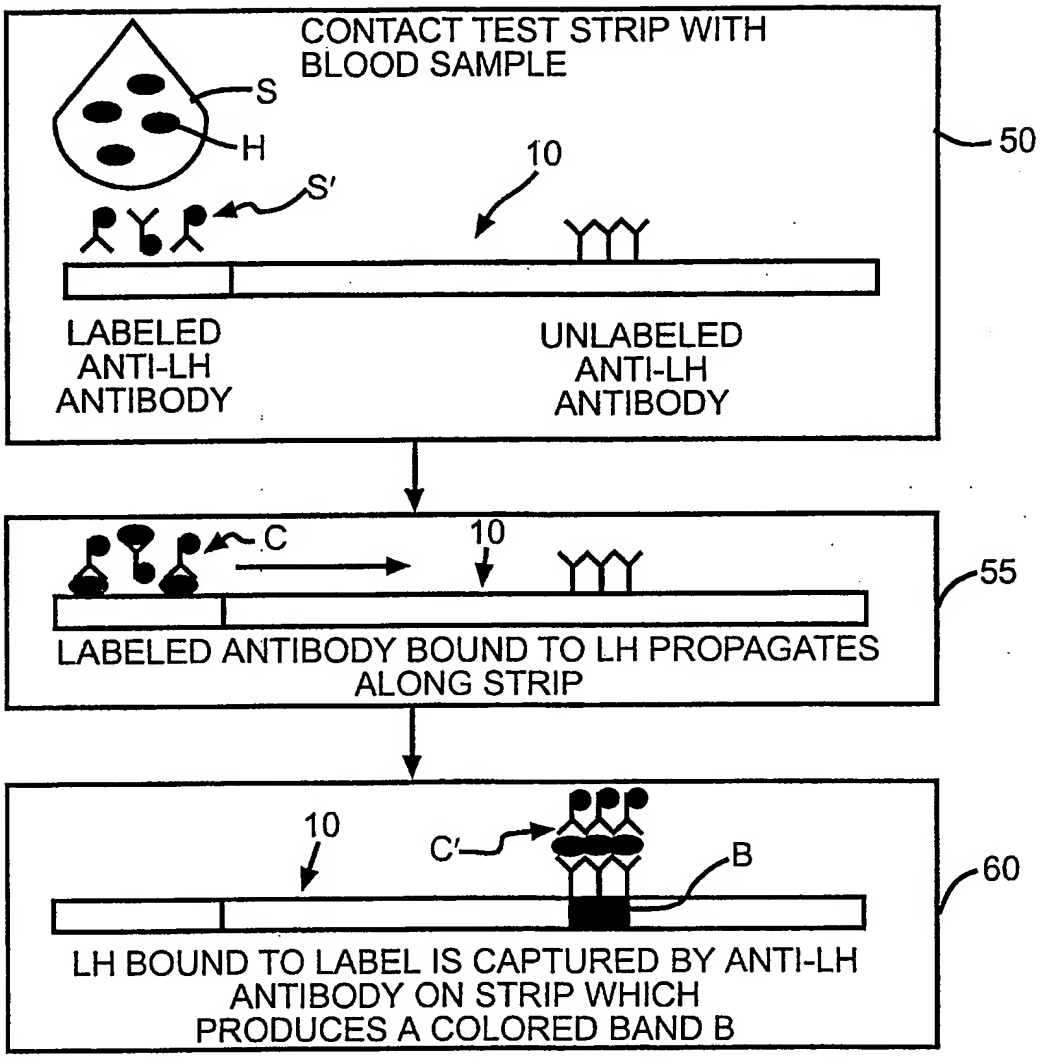
CONTACT TEST STRIP WITH BLOOD SAMPLE
S
H
S'
10
50
LABELED ANTI-LH ANTIBODY
UNLABELED ANTI-LH ANTIBODY
C
10
55
LABELED ANTIBODY BOUND TO LH PROPAGATES ALONG STRIP
10
C'
B
60
LH BOUND TO LABEL IS CAPTURED BY ANTI-LH ANTIBODY ON STRIP WHICH PRODUCES A COLORED BAND B

FIG. 2

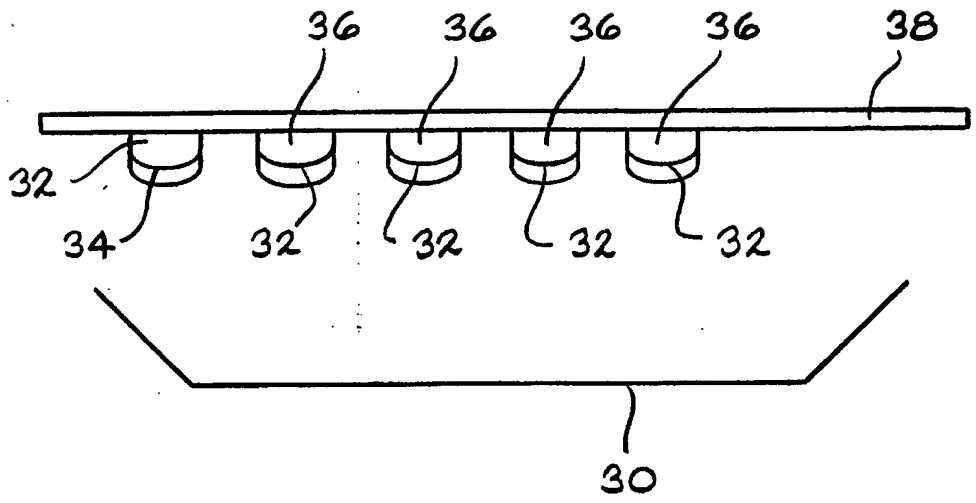

FIG.3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/22614

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 33/48, 33/53; C12M 1/00, 1/40

US CL : 436/65, 814, 906; 435/7.1, 287.3, 287.4, 288.7, 806, 970

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/65, 814, 906; 435/7.1, 287.3, 287.4, 288.7, 806, 970

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN- MEDLINE AND BIOSIS
WEST
EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,602,040 A (MAY et al) 11 February 1997, column 1, line 36 to column 7, line 57.	1-34
X, P	US 5,874,216 A (MAPES et al) 23 February 1999, column 6, line 62 to column 8, line 13.	1-44
Y	US 5,141,850 A (COLE et al) 25 August 1992, column 2, line 23 to column 4, line 6.	1-44
Y	US 5,075,078 A (OSIKOWICZ et al) 24 December 1991, column 5, line 1 to column 6, line 11.	1-18
Y	US 5,559,041 A (KANG et al) 24 September 1996, column 2, line 45 to column 10, line 45.	1-44

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

15 DECEMBER 1999

Date of mailing of the international search report

04 FEB 2000

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/22614

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I. Claims 1-36, drawn to a process and an apparatus for determining ovulation.

Group II. Claims 37-44, drawn to a method for determining ovulation.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group II contains a materially different method of determining ovulation then the method included in Group I. Group II further specifies a comparison step with colormetric chart as well as additional blocking reagent in a test strip matrix.